§ 620.43 Reference BCG Vaccine.

A reference BCG Vaccine, for use in determining the validity of the test for colony-forming units, is to be obtained from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

§ 620.44 Potency tests.

(a) *Colony-forming units (CFU).* The number of CFU must be determined on the contents of each of at least 10 individual final containers of each lot of BCG Vaccine. Of the 10 or more individual final containers, the contents of at least 5 before, and an equal number after, freeze-drying must be tested. Final containers of the freeze-dried vaccine are to be reconstituted as for human use with the diluent recommended by the manufacturer. The number of CFU to be reported for each lot of BCG Vaccine must be determined only from test tubes containing between 10 and 50 CFU. Dilutions must be made as follows:

(1) Dilutions are made from an appropriate volume of the liquid vaccine before freeze-drying or the reconstituted vaccine after freeze-drying. Appropriate dilutions are made with modified Youman's medium specified in paragraph (a)(4) of this section, up to a point where subsequent serial half-log dilutions will result in at least 1 tube containing between 10 and 50 CFU.

(2) Serial half-log dilutions are made in 16×125 millimeter screw-capped test tubes into which 4.5 milliliter aliquots of the diluent prescribed in paragraph (a)(4) of this section have been dispensed. Two milliliters of thoroughly mixed vaccine are added to the first tube of the half-log series, mixed thoroughly, and 2.0 milliliters from this tube are transferred to the next tube in the series. The process of mixing and serially transferring 2.0 milliliters is repeated through each consecutive tube and 2.0 milliliters are discarded from the last tube.

(3) After the serial half-log dilutions are completed, 0.5 milliliter of 1.5 percent agar solution that has been cooled to 42° C is quickly added, where necessary, to make a final concentration of 0.15 percent agar, and the contents of the tubes are thoroughly mixed. After mixing, all tubes are incubated at 35° to 37° C for 3 to 4 weeks.

(4) The composition of modified Youman's medium with bovine albumin is as follows:

Asparagine	5.0 grams.
Monopotassium phosphate (KH_2PO_4).	Do.
Potassium sulfate (K_2SO_4)	0.5 grams.
Magnesium citrate	1.5 grams.
Monosodium glutamate	19.0 grams.
Glycerine	20.0 milliliters.
Distillled water q.s. to	900.0 milliliters.

One hundred milliliters of 5-percent aqueous solution of bovine albumin that has been sterilized by filtration are added to the Youman's medium to produce a final concentration of 0.5 percent of bovine albumin. The pH is adjusted to 7.0 with 5*N* sodium hydroxide.

(b) *Intradermal guinea pig test.* Two or more guinea pigs, each weighing no less than 250 grams, must be injected intradermally in 4 different sites with the following amounts and dilutions of each lot of BCG Vaccine:

(1) Vaccine intended for intradermal injection is reconstituted as for human use with the diluent recommended by the manufacturer. One-tenth milliliter of reconstituted vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) of the reconstituted vaccine are injected into the guinea pigs. The diluent for the ten-fold dilutions is isotonic solution for injection.

(2) Vaccine intended for percutaneous injection into humans is reconstituted with the diluent recommended by the manufacturer so that at least one human dose (estimated to be within a range of from 1 to 33×10^5 CFU) is contained in 0.1 milliliter. A narrower range of CFU is determined for each specific vaccine by the manufacturer and specified in the license application. One-tenth milliliter of the selected dose of vaccine and 0.1 milliliter each of three ten-fold dilutions (1:10, 1:100, and 1:1000) are injected into the guinea pigs. The diluent for the ten-fold dilutions is an isotonic solution for injection.

(3) The lot of vaccine is satisfactory if: